

CLAIMS

1. A bacterial autoinducer, characterised in that it has substantially the following properties:

- Sub-B8
- i) it is produced in response to noradrenaline in serum SAPI medium;
 - ii) it is heat stable;
 - iii) it is stable to lyophilisation;
 - iv) it has a negative charge;
 - v) it is polar;
 - vi) it is hydrophilic;
 - vii) it will not partition into organic solvents;
 - viii) it is capable of binding positively charged metal ions; and
 - ix) it has a molecular weight of about 300-1500 daltons

2. A bacterial autoinducer according to claim 1, further characterised in having at least one of the following characteristics:

- i) it has absorbion maxima at 255,325 and 500-550nm; and
- ii) it is stable in prolonged storage in a dried state and/or in solution.

3. A bacterial autoinducer according to ^{claim 1} ~~either one of claims 1 or 2~~, further characterised in having at least one of the following characteristics:

- i) it is produced in substantially smaller quantities by bacteria grown in LURIA broth, Tryptone soya broth, M9 minimal medium and Davis-Mingioli/minimal medium than by the same bacteria grown in serum SAPI medium;
- ii) it has a reddish-pink colour, reversibly decolorisable by reducing the pH to <4;
- iii) it contains serine;
- iv) its synthesis involves the entA and entB gene products;

- Sub.B8
- v) its synthesis is not stimulated by conditions of Fe starvation;
 - vi) it is synthesised in conditions of excess Fe;
 - vii) its entry into bacteria occurs via a tonB dependent receptor;
 - viii) it is inactivated by oxidation;
 - ix) it is inactivated by extreme pH; and
 - x) it is resistant to degradation by ribonuclease, deoxyribonuclease, trypsin, pepsin, V8 protease, proteinase K, acid phosphatases, alkaline phosphates and phosphodiesterase.

claim 1

4. A bacterial autoinducer according to ~~any one of the preceding claims~~, being an *E.coli*, *Hafnia alvei* or *Salmonella* autoinducer

5. A method for isolating and purifying a bacterial autoinducer, comprising the steps of:

- i) collecting a sample containing the autoinducer;
- ii) fractionating the sample to isolate fractions corresponding to molecular weights of approximately 300-1500 Daltons; and
- iii) eluting the isolate of (ii) on an anion-exchange chromatographic column and selecting the fraction containing the autoinducer.

6. A method according to claim 5, comprising the additional step of concentrating the sample prior to fractionating.

7. A method according to claim 6, concentration being achieved by passing the sample through an approximately 0.2 μ m diameter filter, lyophilising the sample and passing it through an approximately 0.2 μ m diameter filter.

8. A method according to claim 6, concentration being achieved by means of ultrafiltration.

9. A method according to claim 8, ultrafiltration being performed with a molecular weight cut-off of approximately 100 Daltons.

10. ^{claim 5}
~~any one of claims 5-9~~ A method according to ~~any one of claims 5-9~~, the sample being collected from a culture containing bacteria and the autoinducer.

11. A method according to claim 10, the sample being a supernatant collected from a centrifuged culture containing bacteria and the autoinducer.

12. ^{claim 5}
~~any one of claims 5-11~~ A method according to ~~any one of claims 5-11~~, size exclusion gel filtration being performed using a buffer of approximately 100 mM ammonium bicarbonate, pH 8.0, anion exchange purification being performed on an anion exchange column and triethylammonium bicarbonate.

13. ^{claim 5}
~~any one of claims 5-11~~ A method according to ~~any one of claims 5-11~~, size exclusion gel filtration being performed using a buffer of approximately 20 mM potassium phosphate containing 150 mM NaCl, pH 7.4, anion exchange purification being performed on an anion exchange column and NaCl gradient.

14. ^{claim 5}
~~any one of claims 5-13~~ A method according to ~~any one of claims 5-13~~, the bacterium from which the autoinducer is derived being *E. coli*, *Salmonella* or *Hafnia alvei*.

15. ^{claim 5}
~~any one of claims 5-14~~ A bacterial autoinducer isolated and purified according to the method of ~~any one of claims 5-14~~.

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